REMARKS

The specification has been amended to reflect the national stage status. In addition, the multiple dependencies of the claims have been eliminated to reduce the PTO filing fee.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version with markings to show changes made**".

Favorable action on the merits is solicited.

Respectfully submitted,

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DESCRIPTI N

A Method for Constructing Recombinant Adenovirus Vector

This application is a 371 of PCT/JP00/04815 filed July 18, 2000.

Technical Filed

The invention of the present application relates to a method for constructing a recombinant adenovirus vector. More specifically, the invention of the present application relates to a method for constructing a recombinant adenovirus vector which is useful for introducing genes into mammalian cells, and genetic engineering materials for said method.

Background Art

In recent years, the life science has made a long stride progress due to the accumulation of knowledge and the development of various technology in the fields of molecular biology, molecular genetics and the like, whereby a number of information for life phenomena can now be obtained. Research and development are being vigorously carried out in various fields, and the analysis of gene functions has a significant weight among such research and development. Specifically, various technology for introducing an isolated gene into cells or organs in vivo, and vectors which are used for such technology, have been developed.

Vectors of a number of types which are used for introducing genes to a mammalian cell have also been developed. In recent years, vectors produced by utilizing virus (virus vectors) are drawing attention. Among the virus vectors, an adenovirus vector, in particular, can be infected not only to dividing cells but also

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CLAIMS

1. A method for constructing a recombinant adenovirus victor having a DNA sequence consisting of an adenovirus genome DNA and an expression cassette, which comprises:

constructing a recombinant cosmid/adenovirus vector by inserting and ligating a cosmid sequence having recombinase recognition sequences at both ends and the expression cassette into a site of the adenovirus genome DNA where E1 region or E1 and E3 regions are deleted;

cotransfecting this recombinant cosmid/adenovirus vector and a recombinase-expression vector into a cell line producing adenovirus E1 protein; and

deleting the cosmid vector sequence from the recombinant cosmid/adenovirus vector in the cells.

2. The method according to claim 1, wherein the recombinase is Cre recombinase and the recognition sequences thereof are loxP sequences.

3. The method according to claim 1, wherein the recombinase is FLP recombinase and the recognition sequences thereof are FRT sequences.

4. (amended) The method according to ~~any of~~ claim~~s~~ 1 ~~to 3~~, wherein the cell line producing adenovirus E1 protein is 293 cell derived from human fetal kidney cells.

5. A method for constructing a recombinant adenovirus victor having a DNA sequence consisting of an adenovirus genome DNA and an expression cassette, which comprises:

constructing a recombinant cosmid/adenovirus vector by inserting and ligating a cosmid sequence having recombinase recognition sequences at both ends and the expression cassette into a site of the adenovirus genome DNA where

E1 region or E1 and E3 regions are deleted;

transfecting this recombinant cosmid/adenovirus vector into a cell line producing recombinase and adenovirus E1 protein; and

deleting the cosmid vector sequence from the recombinant cosmid/adenovirus vector in the cells.

6. The method according to claim 5, wherein the recombinase is Cre recombinase and the recognition sequences thereof are loxP sequences.

7. The method according to claim 5, wherein the recombinase is FLP recombinase and the recognition sequences thereof are FRT sequences.

8. (Amended) The method according to ~~any of~~ claim~~s~~ 5 ~~to 7~~, wherein the cell line producing recombinase and adenovirus E1 protein is 293 cell derived from human fetal kidney cells which produces the recombinase.

9. A cosmid/adenovirus vector, which comprises a cosmid sequence having recombinase recognition sequences at both ends in a site of the adenovirus genome DNA where E1 region or E1 and E3 regions are deleted.

10. The cosmid/adenovirus vector of claim 9, wherein the recombinase is Cre recombinase and the recognition sequences thereof are loxP sequences.

11. The cosmid/adenovirus vector of claim 9, wherein the recombinase is FLP recombinase and the recognition sequences thereof are FRT sequences.

12. A 293 cell line derived from human fetal kidney cells, which produces FLP reccombinase.